Digestion and Metabolism of Carbohydrates in Fish

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Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2014

Acta Universitatis agriculturae Sueciae
2014:1

Cover: Arctic charr, Tilapia and Eurasian Perch
(Photography: Aleksandar Vidakovic)

ISSN 1652-6880
ISBN (print version) 978-91-576-7950-5
ISBN (electronic version) 978-91-576-7951-2

Print: SLU Service/Repro, Uppsala 2014

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Abstract

This thesis deals with the digestion and metabolism of carbohydrates in Arctic charr, Eurasian perch and tilapia. Two sources of carbohydrates, native starch (wheat) and chitin (zygomycete biomass), were evaluated.

Gut tissue of Arctic charr displayed significant chitinase activity, of both endo- and exo-chitinase forms. Moreover, the distribution pattern along the gastrointestinal tract of Arctic charr differed between endo-chitinase and exo-chitinase. The endo-chitinase activity in stomach tissue and in the distal intestine was several hundred-fold higher than the exo-chitinase activity in stomach tissue. The greatest exo-chitinase activity was found in the distal intestine fed a zygomycete-based diet. Disturbed intestinal integrity and increased uptake rate of the amino acid lysine were observed in the distal, but not proximal, intestine of fish fed the zygomycete-based feed.

A ^{1}HNMR metabolomics approach revealed no differences in metabolic profile in liver tissues of Arctic charr fingerlings fed a zygomycete-based diet and a fish-meal based diet.

The inclusion of wheat starch did not affect α-amylase activity in gut tissue of Arctic charr and Eurasian perch. Overall, α-amylase activity was correlated with the trends obtained for starch digestibility. The apparent digestibility (AD) of crude protein, starch, crude fat and energy differed between the fish species, with on average higher values for all parameters in Eurasian perch than in Arctic charr. Within fish species, dietary starch level had no effect on AD of dry matter, crude protein, crude fat and energy.

Studies of the metabolic response to wheat starch inclusion in Arctic charr and tilapia using ^{1}HNMR base metabolomics indicated metabolic effects in tilapia, while inclusion of starch in the diet of Arctic charr resulted in partial or negligible metabolism effects. Thus there are species-related differences in the metabolic response to dietary starch inclusion.

Keywords: Carbohydrates, chitin, metabolism, fish, diet, metabolites, wheat starch, enzyme, zygomycete

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Dedication

To my Parents, Husband Shahid,
Little Princess Samreen and Little Prince Ayan
for their Endless Love

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Abro, R., Sundell, K., Sandblom, E., Sundh, H., Brännäs, E., Kiessling, A., Lindberg, J. E., Lundh, T. (2013). Zygomycete biomass in the diet enhances intestinal chitinolytic activities and increases leakiness in the intestinal epithelia of Arctic charr (*Salvelinus alpinus*). *Comparative Biochemistry and Physiology B* (Submitted).

II Abro, R., Moazzami, A. A., Lindberg, J. E., Lundh, T. (2013). Metabolic insights in Arctic charr (*Salvelinus alpinus*) fed zygomycetes and fish meal diets as assessed in liver using nuclear magnetic resonance (NMR) technology. *International Aquatic Research* (Submitted).

III Abro, R., Moazzami, A. A., Lindberg, J. E., Lundh, T. (2013). NMR-based metabolomics reveals dietary effects in liver extracts of Arctic charr (*Salvelinus alpinus*) and tilapia (*Oreochromis mossambicus)* fed different levels of starch (Manuscript).

IV Abro, R., Lundh, T., Lindberg, J. E. (2013). Effect of dietary starch inclusion rate on digestibility and amylase activity of Arctic charr (*Salvelinus alpinus*) and Eurasian perch (*Perca fluviatilis*). *Journal of Aquaculture Research and Development* [*5: 209 doi:10.4172/2155-9546.1000209*].

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Abbreviations

AD	Apparent digestibility
ADC	Apparent digestibility coefficient
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
CP	Crude protein
DM	Dry matter
EE	Ether extract
FM	Fish meal
FZ	Fish meal containing zygomycete biomass
GE	Gross energy
GI	Gastrointestinal
GlcN	Glucosamine
GlcNAc	N-acetylglucosamine
MS	Mass spectrometery
NMR	Nuclear magnetic resonance
OPLS-DA	Orthogonal partial least squares-discriminate analysis
Papp	Paracellular permeability
PCA	Principal component analysis
RIA	Radio immunoassay
SCC	Short circuit current
ST	Standard
TEP	Transepithelial potential
TER	Transepithelial resistance
UDP	Uridine diphosphate
WS	Wheat starch

1 Introduction

Aquatic food products are an integral part of the human diet in many parts of the world and significantly contribute to the supply of high quality protein. Fish and fish products are obtained by fishing and by cultivation in available water resources. Global annual aquaculture production has been increasing at an average rate of 2.9% during the past four decades (FAO–FISHSTAT, 2012). Feed supply and feed costs are amongst the greatest challenges for the development of sustainable fish farming. Therefore, the aquaculture industry is searching for feed ingredients that can be used to formulate cheap fish feed (Stone, 2003). It was estimated that fish meal and fish oil contribute 75% of the protein and 35% of the energy in aquaculture feed (Tacon, 1999). The increasing costs and demand for fish meal and fish oil are a particular obstacle to achieving a long-term sustainable increase in fish production, so alternative feed sources that can replace fish meal and fish oil are required (Kristofersson & Anderson, 2006). These alternative feed sources possible to use can be of varying types and originate from plants, animals or microbes (Camacho-Rodriguez *et al.*, 2013; Slawski *et al.*, 2013; Wang *et al.*, 2013; Yun *et al.*, 2013). Carbohydrates are often cheaper dietary energy sources than protein and lipids. However, fish species show different ability to digest and metabolise alternative dietary components, in particular the carbohydrate fraction (Dabrowski & Guderley, 2002; Hemre *et al.*, 2002). The digestion and metabolism of feed ingredients is dependent on fish species and on the source, inclusion level and treatment of the ingredient (Krogdahl *et al.*, 2005; Stone, 2003). Knowledge of the capacity to utilise carbohydrates in the diet is an essential pre-requisite for appropriate formulation of fish feed (Wilson, 1994).

This thesis focuses on digestion and metabolism of carbohydrates from cereals, in the form of wheat starch, and from micro fungi, in the form of chitin. Both sources were used in unprocessed form in order to assess the

capacity of the various fish species studied to utilise the native forms of the two carbohydrates.

2 Background

The human population is continually increasing world-wide and is projected to reach 10-12 billion people by 2050 (Welch & Grahm, 1999). This poses major challenges to increase food production in order to feed the growing population. In this context, the aquaculture sector has great potential, as it can provide nutritious and high-quality food products for humans (Diana, 2009).

2.1 Aquaculture production in the Nordic countries

Commercial rearing of Arctic charr (*Salvenius alpinus*), Atlantic salmon (*Salmo salar*), European eel (*Anguilla Anguilla*), Eurasian perch (*Perca fluviatilis*), rainbow trout (*Oncorhynchus mykiss*), pike perch (*Stizostedion lucioperca*), Nile tilapia (*Oreochromis niloticus*), sturgeon (*Acipenseriformes*) and European lobster (*Homarus gammarus*) is increasing in the Nordic countries (Dalsgaard *et al.*, 2013). The natural environmental and ecological conditions adversely influence the aquaculture industry in the Nordic countries (Martins *et al.*, 2010). Therefore, production of cost-effective feed for the commercial rearing of fish is essential for sustainable aquaculture in this part of Europe.

2.2 Fish feed

The cost of aquaculture production can be reduced by efficient feed formulation (Ganguly *et al.*, 2013). Fish have different dietary requirements and a varying capacity to utilise available feed resources, largely determined by their natural feeding habitats (Glencross *et al.*, 2007). The current diets for most farmed fish are based mainly on fish meal (Gatlin *et al.*, 2007). Fish meal is the main dietary protein source in aquafeeds and was estimated to constitute 20-60% of fish diets (Glencross *et al.*, 2007; Watanabe, 2002). However, the

availability of fish meal is limited and it is an expensive component of formulated aquafeeds (Gatlin *et al.*, 2007). Therefore, fish meal is the major constraint for long-term sustainable development of aquaculture production.

2.2.1. Feed and feeding practices in Arctic charr

Arctic charr is a carnivorous, cold water salmonid fish well known for its quality of texture and taste (Cyprian *et al.*, 2008). Arctic charr shows rapid growth in fresh water conditions and has great potential for commercial production (Wandsvik & Jobling, 1982; Gjedrem & Gunnes, 1978). Generally, the dietary protein requirements are reported to be similar to those of other salmonids (Jobling & Wandsvik, 1983). High growth rates have been achieved on diets containing 44-54% protein and 20% lipid in Arctic charr (Tabachek, 1986).

2.2.2. Feed and feeding practices in Eurasian perch

Eurasian perch (*Perca fluviatilis*) is a carnivorous fish and has been recognised as a promising aquaculture candidate (Kestemont & Mélard, 2000). In most cases, the feed formulated and designed for perch has been similar to that used for salmon, trout or sea bass (Fontaine *et al.*, 1997). However, these diets have failed to meet the nutritional requirements of Eurasian perch (Melard *et al.*, 1996). Generally, it is believed that perch has the potential and ability to metabolise carbohydrates efficiently (Borrebaek & Christophersen, 2000). For example, it has been reported that perch has the ability to utilise dietary carbohydrates (20-40%) and fibre (15%) (Allan *et al.*, 2000). However, the utilisation and digestion abilities in silver perch could be influenced by type, source and physical state of dietary carbohydrates (Stone *et al.*, 2003).

2.2.3. Feed and feeding practices in tilapia

Tilapia is an omnivorous, warm water fish widely distributed in many countries around the world (Trewavas, 1983). It has been reported that tilapia has greater potential to utilise starch than carnivorous fish species (Krogdahl *et al.*, 2005). Several studies have reported that an increase in dietary carbohydrate content improves metabolism and growth in tilapia (Azaza *et al.*, 2013; Shiau, 1997; Tung & Shiau, 1993). Improved growth was observed in tilapia fed diets with 10-40% inclusion of starch (Amirkolaie *et al.*, 2006; Anderson *et al.*, 1984).

2.3. Protein sparing effect of carbohydrates

Protein is usually the most expensive ingredient in the formulated aquafeed. Thus, feed production and utilisation per unit cost is of highly significant in the development of an economically sound aquafeed. Carbohydrates are easily available and inexpensive sources in formulated feed which are efficiently utilised in several fish species (Zhao *et al.*, 2011; Gao *et al.*, 2010). Carbohydrates are of great value as they have protein sparing effects in salmonid and tilapia fish species (Azaza *et al.*, 2013; Hemre *et al.*, 1995). On the other hand, nutrient requirements are specific for different fish species with respect to protein and carbohydrates (Wilson, 1994).

2.4. Alternative ingredients in fish feed

An adequate and long-term sustainable feed supply is a critical for fish culture. Moreover, the cost of feed ingredients is of major concern, as it constitutes the greatest production cost (40-60%) (Gatlin *et al.*, 2007). Most commercial fish feeds contain fish meal (30-70%) as a major source of protein (Rumsey, 1993). The high cost of fish meal used in aquafeeds necessitates its replacement with cheaper alternative feed ingredients. In recent studies some conventional, widely available alternative dietary ingredients such as lupin kernel meal (Molina-Povedaa *et al.*, 2013; Zhang *et al.*, 2012b) soybean meal (Rossi *et al.*, 2013) yeast extract (Trosvik *et al.*, 2013), corn germ meal (Li *et al.*, 2013a), sea cucumber meal and canola protein (Slawski *et al.*, 2013) have been tested in various cultured fish species. It was shown that these feed ingredients could successfully be used in formulated aquafeed as a replacement for fish meal. However, these ingredients are also used for human and farm animal consumption. With this in mind, use of carbohydrate by-products such as spent sulphite liquor from the paper pulp industry in the formulation of aquafeed would be of great value as a renewable resource for sustainable aquaculture production (Kiessling, 2009). It has been shown that high biomass yield can be achieved by cultivation of the fungus *Rhizopus oryzae* on paper pulp spent sulphite liquor (Taherzadeh *et al.*, 2003).

2.4.1. Carbohydrates

Carbohydrates are the main source of energy in most animal diets and are classified based on the constituent sugars, structure, composition, degree of polymerisation and glycosidic linkage into *e.g.* non-monomer carbohydrates such as oligosaccharides (lactose, maltose), polysaccharides (starch, chitin, cellulose) and monomer sugars (glucose, fructose) (Englyst & Hudson, 1996). Carbohydrate properties, such as digestion and absorption rate, viscosity,

structural features, water-binding capacity and fermentation ability in the GI tract, are of vital importance for their nutritional effects (Asp, 1996). Starch is an energy storage nutrient in wheat and constitutes approximately 60% of the total grain (Novus, 1992) and is composed of glucose molecules linked together by α-glycosidic bonds and this linkage of glucose units influence the enzymatic activities in fish (Smith, 1989). Dietary carbohydrate inclusion in the several fish species appears to produce positive effects on growth and digestibility (Li *et al.*, 2013b; Hung *et al.*, 2003; Watanabe, 2002). However, using the appropriate level of carbohydrates in aquafeeds is of great importance, because if the appropriate amount of carbohydrates is not provided, this may have negative effects on nutrient utilisation, growth, metabolism and health (Li *et al.*, 2012; Erfanullah & Jafri, 1998).

2.4.2. Chitin

Chitin is a heteropolysaccharide comprising β-(1-4) linked N-acetylglucosamine molecules and is the second most abundant compound found around the globe (Flach *et al.*, 1992). This polymer is found and synthesised in various living organisms (Rinaudo, 2006; Kumar, 2000). Naturally, chitin contained as a supporting material in arthropods, fungi, yeasts, sponges, sea corals, crustaceans, crabs, shrimp, lobster, krill and prawn (Mathur & Narang, 1990). Waste production in the form of crustacean shells and other aquatic waste is estimated to approximately 1.2×10^6 tons annually, which is a major environmental concern (Knorr, 1991). It has been reported that inclusion of chitin in aquafeed results in improved growth rate in some fish species (Tibbetts & Lall, 2013; Harikrishnan *et al.*, 2012). Moreover, supplementation of chitin or chitosan in the diets of kelp grouper and orange-spotted grouper fish enhances the immune response and affords disease resistance against pathogens (Harikrishnan *et al.*, 2012; Zhang *et al.*, 2012a).

2.5. Utilisation of carbohydrates

Fish have to adopt a range of strategies for coping with food deprivation or variations in their natural diet. In this context, the different fish species display metabolic patterns which meet their dietary requirements (Bellamy, 1968). Digestion and metabolism of dietary carbohydrates varies and depends upon several factors in addition to fish species, such as environmental conditions, and type and source of carbohydrates (Hutchins *et al.*, 1998; Grisdale-Helland & Helland, 1997; Gaylord & Gatlin, 1996). It is generally believed that warm water fish species utilise carbohydrates more efficiently and at higher levels

than cold water and marine fish species (Wilson, 1994). Omnivorous fish species such as Nile tilapia and common carp, which feed at low trophic levels, can efficiently utilise high dietary levels of carbohydrates (30-50%) in comparison to the high trophic level carnivorous fish species (Enes *et al.*, 2011; Enes *et al.*, 2006; Hemre *et al.*, 2002; Wilson, 1994). In fact, no particular dietary carbohydrate level has been defined for fish. However, some carbohydrates must be supplied in aquafeed in order to maintain the normal growth of fish (Peragon *et al.*, 1999). Interestingly, fish have similar carbohydrate metabolic pathways as other mammals, but lower carbohydrate tolerance than mammals (Moon, 2001).

2.5.1. Digestibility

The nutritive value of the feed depends on the digestibility of each ingredient in the diet, but also on the interactions among ingredients (Alexis, 1990). The digestibility of starch varies in fish species in the dietary levels. For instance, in Atlantic halibut the digestibility of starch decreases from 84 to 53% when the dietary inclusion level is increased from 8 to 17% (Grisdale-Helland & Helland, 1998). Moreover, some fish species show reduced growth rates when fed carbohydrate-free diets (Wilson, 1994). Digestible efficiency of digestible and non-digestible carbohydrates varies in herbivorous and carnivorous fish species (Panserat *et al.*, 2009; Krogdahl *et al.*, 2005). The herbivorous fish species can utilise part of the non-starch carbohydrates in the diet due to symbiosis with the gut microbiota. However, most fish species are unable to utilise non-starch carbohydrates properly because of lack of adequate gut microbiota for their digestion (Krogdahl *et al.*, 2005).

Feed ingredient digestibility can be assessed by two techniques, *i.e*. the direct method and the indirect method. In the direct method, total feed consumed and faeces voided from the fish are quantified (NRC, 1993; Smith, 1971). The method can be used in determining digestible energy, metabolisable energy and carbon and nitrogen balance (NRC, 1993). The indirect method is involving the use of a non-digestible marker (*e.g*. chromium oxide or titanium dioxide) and spot-sampling of excreta to measure the digestion coefficients for energy and dietary components (NRC, 1993; Cho *et al.*, 1982).

2.5.2. Enzymology

Enzymes are protein in nature and comprise biological molecules which are involved in metabolic processes in living organisms (Grisham & Reginald, 1999). The efficiency of feed utilisation depends on physiological capacity to digest and transform ingested nutrients (Furne *et al.*, 2008). The digestion and

absorption of nutrients are mostly dependent on enzyme activities involved in breakdown and assimilation of food (Klein *et al.*, 1998). Therefore, analysis of enzyme activities is a convenient and reliable technique that can provide comprehensive information relating to digestive physiology and nutritional conditions in the fish (Bolasina *et al.*, 2006). The information obtained can be helpful for the design of feeding strategies and formulation of fish diets (Verreth & Segner, 1995). Digestive enzyme activities in fishes are associated with feeding ecology and composition of diet (Fernandez *et al.*, 2011). In general, herbivorous fish species possess greater carbohydrate enzyme activity, while carnivorous fish species exhibit higher proteolytic enzyme activity (Hidalgo *et al.*, 1999).

2.5.2.1. Amylase

The main digestive enzymes involved in the metabolism of starch α-amylase and α-glucosidase. These enzymes hydrolyse the α-glycoside linkage of starch to produce glucose (Mizutani *et al.*, 2012; Fernandez *et al.*, 2011; Kuzmina, 1996). Usually, amylase activity is varies with different developmental stages of fish. Amylase is synthesised in the pancreas and is secreted into the gut (Fish, 1960), where most of the enzyme is found, and absorbed into the mucosa of the intestine and the pyloric ceca (Munillamoran & Stark, 1990; Ugolev *et al.*, 1983). The actual amylase activity generally depends on the natural diet of the different fish species (Hidalgo *et al.*, 1999; Hofer *et al.*, 1982). However, amylase activity can be influenced by degree of filling of gut, nutritional status, temperature and adaptive mechanisms induced by the diet (Kuzmina, 1996; Bitterlich, 1985; Takii *et al.*, 1985). Generally, omnivorous fish species possess higher amylase activity than carnivorous fish. For example, carp, goldfish and trench show higher amylase activity than seabream, eel and trout (Hidalgo *et al.*, 1999). It has been reported that inclusion of dietary starch result in increase in amylase activity in sea bass and yellow croaker (Yu *et al.*, 2012; Peres *et al.*, 1996).

2.5.2.2. Chitinases

Chitinolytic enzymes are involved in degradation of chitin into oligomers composed of N-acetyl-glucosamine. Chitinase plays a significant role for the digestion of chitin containing food in fish (Gutowska *et al.*, 2004) and host defence against chitin-coated pathogens (Okada *et al.*, 2013; Zhang *et al.*, 2013). In vertebrates, chitinase is found in various organs such as stomach, intestine, spleen, kidney and macrophage cells (Ikeda *et al.*, 2013; Lindsay *et al.*, 1984). In fish stomach, chitinases are involved in the degradation of chitin and prevention of fragment blockage in the intestine (Lindsay *et al.*, 1984).

Chitinases are comprised of two main groups, *i.e*. endo-chitinase and exo-chitinase (Nord & Wadstrom, 1972; Wadstrom, 1971). Endo-chitinase is involved randomly catalysis the breakdown of chitin to produce chitin to produce chitin oligosaccharides (Ikeda *et al.*, 2009; Kang *et al.*, 1999). Exochitinase is involved in the cleavage of chitin into monomers of N-acetylglucosamine (Kang *et al.*, 1999).

2.6. Gut physiology

Gut functions in animals are of vital importance for their performance, health and survival. The physiology of each gut region must functional properly in order to maintain food digestion and absorption processes (Jutfelt, 2011). Intestinal barriers are involved in preventing the penetration of dietary components, allergens and pathogens into the mucosa (Jutfelt, 2011; Martin-Venegas *et al.*, 2006). Therefore, maintaining the intact integrity of the primary barriers is essential for healthy fish production and reduced infection susceptibility. Harmful diet components can lead to adverse effects on intestinal barrier functions and local inflammation (Knudsen *et al.*, 2008; Jutfelt *et al.*, 2007).

Intestinal barrier functions can be assessed using the Ussing chamber, which is a standard technique to investigate secretion and absorption of ions and intestinal physiology. The method is a valuable tool for the quantitative and qualitative analysis of transportation of various nutrients and ions across the intestinal epithelium (Wright, 1993; Stevens, 1964).

2.7. Metabolomics

Metabolomics has emerged as a developing discipline that deals with chemical and biological processes associated with metabolites. The discipline provides a promising and valuable tool for high-throughput identification and quantification of several metabolites in biological systems, with high accuracy and quality in comparison to traditional approaches used in past decades (Dunn & Hankemeier, 2013; Dunn *et al.*, 2013).

Two highly sophisticated techniques, *i.e*. mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectrometry are currently being applied in the field of metabolomics research. The techniques can be used to identify the thousands of metabolite(s) of interest in the sample, with limited or without prior information of composition of the sample (Dunn *et al.*, 2013). The techniques permit accurate identification of small molecules (<1500 Da) in biological samples that can be related to nutritional interventions (Wishart *et*

al., 2007; Moco *et al.*, 2006) and provide information that is not possible to achieve with previous traditional methods. NMR is being extensively applied in food analysis and food processing (Marcone *et al.*, 2013), *e.g.* for edible oil (Barison *et al.*, 2010), fish (Wagner *et al.*, 2014; Nestor *et al.*, 2010), beef (Pereira *et al.*, 2013), milk (Maher *et al.*, 2013), cheese (Mulas *et al.*, 2013), tomatoes (Iglesias *et al.*, 2014), coffee (Wei *et al.*, 2012) and bread (Sivam *et al.*, 2013). However, the high costs of instrumentation and lower sensitivity are the major concerns of the ^{1}HNMR approach (Marcone *et al.*, 2013; Sitter *et al.*, 2006).

3 Aims of the thesis

The overall objective of this thesis was to investigate the digestion and metabolism of carbohydrates in Arctic charr, tilapia and Eurasian perch. This was achieved through performing *in vivo* experiments with fish fed different diets, and by collecting faecal and tissue specimens for analysis of enzyme activities, digestibility characteristics, gut function and concentration of metabolites.

The specific aims were:

- To investigate the ability of Artic charr to utilise chitin/chitosan rich diets through measuring chitinolytic activity and to evaluate the effect of inclusion of dietary zygomycetes on intestinal primary barrier function in Arctic charr.
- To compare the metabolic finger-prints in the liver of Arctic charr fed a fish meal-based diet, a zygomycete-based diet and a commercial diet.
- To evaluate the metabolic responses in Arctic charr to inclusion of native wheat starch in the diet with tilapia as a reference.
- To investigate the effect of diets containing different levels of native wheat starch on digestibility and amylase activity in Arctic charr and Eurasian perch.

4 Materials and Methods

4.1 Experimental design

Four experiments were performed, with the different diets fed to triplicate groups (Papers I, II and III) and quadruplicate groups (Paper IV) of fish. Before the start of each experiment, the fish were acclimatised. At the start and end of the experiments, the fish were individually weighed. The numbers of the fish were equally distributed into tanks for each treatment in the different experiments. The fish were randomly allocated to the experimental diets (two diets in Paper I; three diets in Papers II and III; six diets in Paper IV).

4.2 Experimental diets

The experimental diets used for studies of chitinase activity, metabolic profile and barrier function were iso-nitrogenous fish meal-based diets, with or without zygomycete biomass (Papers I and II) and a diet standard commercial (ST) (Skretting Nutra Parr) (Paper II). Three iso-nitrogenous diets containing 0, 10 and 20 % of native wheat starch were formulated to investigate the metabolic profiles in Arctic charr (*Salvelinus alpinus*) and tilapia (*Oreochromis mossambicus*) (Paper III). Six diets containing 0 (control), 10, 15, 20, 25 and 30 % of native wheat starch were formulated for assessment of digestibility and amylase activity in Arctic charr and Eurasian perch (*Perca fluviatilis*) (Paper IV).

4.3 Fish rearing

Arctic charr fingerlings (initial body weight 105 ± 0.5 g in Paper I and 97 ± 22 g in Paper II) were reared at a water temperature of 6 ± 1^{o}C. Fingerlings of tilapia (initial body weight 15 ± 0.5 g) were reared at a water temperature of 28

±1 ^{o}C (Paper III). Fingerlings of Arctic charr (initial body weight of 86 ± 7 g) were reared at a water temperature of 10 ± 1^{o}C (Paper III). Fingerlings of Eurasian perch (initial body weight of 190 ± 0.5 g) were reared at a water temperature of 21 ± 1^{o}C (Paper IV). Arctic charr fingerlings (initial body weight of 102 ± 7 g) were reared at a water temperature of 10 ± 1^{o}C (Paper IV). The fish were fed twice daily with a total daily allowance of 2% of body weight during the experiment. The fish accepted the experimental diets and no mortality were observed during the entire experiments.

4.4 Sample collection

At collection of tissue specimens for analysis, the fish were anesthetised with Tricane methane sulphonate (MS-222) solution (50 mg/L) and killed with a sharp blow to the head. The tissues (liver, stomach, pyloric caeca, proximal and distal intestine) were dissected from the fish and immediately transferred into liquid nitrogen (Papers I-IV). All liver tissue samples were kept frozen at -80 ^{o}C for further NMR (nuclear magnetic resonance) analyses. The distal intestine was dissected and the faecal material collected (Paper IV). A pooled sample from each tank was freeze-dried, finely ground, and stored at -80 ^{o}C for further analyses.

4.5 Determination of enzyme activity

4.5.1 Chitinase activity

Chitinase activity in gut samples was determined using chitin from crab shell (Sigma # C9752) and 4-nitrophenyl N-acetyl-β-D-glucose aminide (Sigma# N9376) substrates (Paper I). The chitinase activity was measured according to the recommendations of the manufacturer (Sigma Chemical Co., St. Louis, Missouri 63103, USA).

4.5.2 Amylase activity

The amylase activity in the proximal and distal intestine of the fish species was measured using the Ceralpha kit (Megazyme K-CERA, Wicklow, Ireland) according to the Ceralpha procedure (Sigma, St. Louis, MO, USA) (Paper IV).

4.6 Biomass analysis

The glucosamine (GlcN) and N-acetylglucosamine (GlcNAc) contents in the experimental diets were measured (Paper I) according to previously described methods (Ferreira *et al.*, 2012; Zamani *et al.*, 2008).

4.7 Ussing chamber experiment

Lysine across the intestinal epithelial tissues was measured using a set of custom made Ussing chambers, especially designed for fish intestines (Sundell *et al.*, 2003). The intestinal segments were mounted into the Ussing chambers and electrical characteristics such as transepithelial potential (TEP), transepithelial resistance (TER), short circuit current (SCC) and transepithelial potential (TEP), were determined as described previously (Sundell & Sundh, 2012).

4.8 NMR analyses

The samples for NMR were prepared (Papers II and III) as described previously (Moazzami *et al.*, 2011; Atherton *et al.*, 2006). The NMR spectrum of liver tissue extracts was determined at 5-mm broad-band probe using a Bruker AV 600 NMR spectrometer (Karlsruhe, Germany). NMR spectra were measured at 298 K with 264 scans and 32,764 data points with a spectral width of 6009.61 Hz. Standard one-dimensional ^{1}HNMR spectra were obtained using water pre-saturation for high peak pulse sequence at 2.72 s (Paper II) / 1.82 s (Paper III) and relaxation delay for 5.0 s (Paper II) /4.0 s (Paper III). NMR spectral data were processed using Bruker Topspin 1.3 software and were Fourier-transformed after multiplication by a line broadening of 0.3 Hz and referred to TSP (sodium-3-(trimethylsily)-2,2,3,3-tetradeuteriopropionate) at 0.0 ppm (Papers II and III). The constituent metabolites were identified and quantified and the data analysed statistically. Metabolic profiling (Paper II) and identification of ^{1}HNMR signals of metabolites were performed using ChenomX software (Evaluation version, ChenomX Inc., Canada).

4.9 Chemical analyses

The chemical composition of feed ingredients was determined using standard methods (Cowey & Froster, 1971). The dry matter (DM) content of feed and faeces was measured by drying at 105^{o}C for 24 h; total nitrogen (N) content was determined using the Kjeldahl method and crude protein (CP) calculated as N x 6.25; fat content was analysed using the Soxhlet method. Ash content was determined using incineration in a muffle furnace at 550 ^{o}C for 12 h. Gross energy (GE, MJ Kg^{-1}) was determined using a bomb calorimeter (Parr 6300, Parr Instrument Company, Molin, IL, USA).

4.10 Statistical analyses

Chitinolytic activity and Ussing chamber data were analysed using three-factorial analyses of variance (ANOVA) in a general linear model (GLM) (SAS, Cary NC, USA)(Paper I). In Paper III, significant metabolite signals were analysed using two-factor orthogonal ANOVA and univariate comparison was performed using Bonferroni correction ($p=0.05$ divided by number of variables). The data on growth performance, digestibility, enzyme activity and metabolic profiles were analysed statistically using the MINITAB® statistical software package (Version 16; Minitab, State College, Pennsylvania) under Proc GLM (Papers I-IV).

5. Summary of results

5.1 Influence on growth performance, chitinolytic activity and intestinal permeability of feeding fish zygomycete based and fish meal-based diets (Paper I)

Body weight of Arctic charr was increased by 15 ± 3% (mean ± SD) during the 4-week experimental period. The fish did not show differences in growth performance between the diets (interaction between diet and time, $p=0.592$). Also, there was no difference observed between fish tanks (interaction between tank and time, $p=0.766$).

The highest exo-chitinase activity was observed in the distal intestinal tissue of fish fed the zygomycete-based diet (FZ) and lowest in the stomach tissues fed diet fish meal-based (FM). Differences in exo-chitinase activity were observed in the GI tract region ($p<0.001$), with greater activity in the distal intestine compared with other GI regions. Overall, there were no significant dietary differences ($P>0.05$) between the two diets on exo-chitinase activity in the gut tissues of Arctic charr. However, there was tendency for an interaction between diet and GI region ($P=0.061$). The highest endo-chitinase activity was noted in stomach and the lowest in proximal intestine of fish fed diet FM. Endo-chitinase activity was not influenced by diet ($P>0.05$). However, an interaction was observed between diet and GI region ($p<0.001$). The endo-chitinase activity in the stomach tissue was higher in fish fed diet FM than diet FZ, whereas no influence was found in the pylorus, proximal and distal intestine.

The paracellular permeability (P_{app}) was similar in the two dietary treatments ($p>0.05$). However, for the factor "region", the permeability of the distal intestine was higher than that of the proximal intestine ($p<0.001$) (Paper I). There was tendency towards an interaction between the factors diet and intestinal region. There was a tendency for reduced TER in the distal intestine

of fish fed diet FZ compared with diet FM ($p=0.06$), while the proximal intestine was unaffected. Multiple post hoc comparison revealed that diet FZ affected P_{app} and promoted lysine transport in the distal intestine ($p<0.05$), whereas there was no dietary effect on lysine uptake in the proximal intestine.

5.2. Metabolic profile in fish fed zygomycete-based and fish meal-based diets (Paper II)

PCA analysis was performed on liver samples collected from Arctic charr fed diets FZ, FM and ST. The PCA score scatter plot with first versus second components exhibited no apparent clustering difference in the different dietary treatments. However, OPLS-DA analysis revealed variation between diets with regard to the pre-defined classes of metabolites. OPLS-DA models were fitted including two diets at each time (FM vs. FZ, FM vs. ST, and FZ vs. ST). The model revealed that diets FM and FZ were not significantly ($p \geq 0.05$) different using CV-ANOVA. However, the other models, *i.e.* FM vs. ST, and FZ vs. ST were found significant using CV-ANOVA ($p < 0.05$). OPLS-DA models indicated that the diet ST apparently separated from the FM and FZ diets.

The comparative analysis of diet FM with diet ST revealed discrimination of metabolites such as acetate, creatine, choline, formate, SN-glycero-3-phosphocholine and an unknown signal were present at higher levels in diet FM than diet ST. Whereas, asparagine was contained in lower level in diet FM than diet ST. The metabolic comparative analysis performed between FZ and ST diets exhibited the discrimination of metabolites, *e.g.* lysine, β-alanine, creatine, formate, glucose, inosine, SN-glycero-3-phosphocholine and an unknown signal were present in higher level in diet FZ than diet ST. Whereas, asparagine and succinate were observed lower level in diet FZ than diet ST.

5.3. Metabolic profile in Arctic charr and tilapia fed diets containing different rates of wheat starch (Paper III)

In tilapia, the PCA analysis revealed there was no clear trend of separation among the clusters on the different diets. In Arctic charr, apparent clustering separation was observed among the dietary starch levels. The PCA score scatter plot indicated that treatments exhibited a clear variation in the clusters related to diets when compared across the fish species. The OPLS-DA score plot showed clear separation among the class components of the diets fed to tilapia and Arctic charr. There were significant interaction observed between treatment and species. In tilapia, the diet containing 20% native wheat starch

resulted in significant effects on the concentration of some metabolites, *e.g.* ADP, creatine, glutamine, glycine UDP-glucuronate and O-phosphocholine. In Arctic charr, significant high concentrations of 3-aminoiso-butyrate, asparagine, alanine and glutamine and lower concentration of choline and glucose were present in 10 and 20 % native wheat starch.

5.4. Effect on amylase activity and digestibility characteristics in Arctic charr and Eurasian perch of feeding graded levels of wheat starch diets (Paper IV)

The AD of DM in Arctic charr was higher with a diet containing 10% wheat starch (diet WS10) than 30% wheat starch (diet WS30) and control diets. In Eurasian perch, the AD of DM was higher in diets WS10 and a diet containing 15% wheat starch (diet WS15) than control diet. There were no significant differences in the AD of CP between graded levels of wheat starch inclusion in the diets of the two fish species. The AD of DM, CP, ether extract (EE), starch and GE differed between the fish species ($p<0.001$), with consistently lower AD values in Arctic charr than in Eurasian perch. The most marked difference in AD between the fish species was observed for wheat starch, where the average AD differed by 21.6% units.

The α-amylase activity was greater in Eurasian perch than in Arctic charr. It was revealed that the proximal region contained higher α-amylase activity than the distal intestine ($p<0.001$). However, within species, α-amylase activity in gut tissues did not differ at different wheat starch inclusion levels.

6. General discussion

6.1. Biomass based diet in relation to growth in Arctic charr

The success of large scale aquaculture farming in the future chiefly be based on the formulation of diets that can be prepared from cheap feed ingredients (Higgs *et al.*, 1995). This thesis focused on the utilisation and metabolism of a renewable natural source of biomass (zygomycete) when included in the diet of Arctic charr (Papers I and II). The findings support those of previous study in Atlantic salmon showing a good growth response when fed a mycelium biomass based diet (Bankefors *et al.*, 2011). Steelhead fish showed significantly higher growth when fed a diet containing chitin than when fed diets based on fish meal, squid meal and canthaxanthin (Lellis & Barrows, 2000). Also, dietary chitin show improved growth response and immune response in ovate pompano (Lin *et al.*, 2012). In fact, it is known that chitin allows protein sparing effects in the diet of juvenile haddock and turbot (Tibbetts & Lall, 2013; Kroeckel *et al.*, 2012) and fry tilapia (Fall *et al.*, 2013). In contrast, dietary supplementation with chitin and chitosan depresses growth in tilapia (Shiau & Yu, 1999). The studies presented in this thesis revealed no significant difference in growth of Arctic charr fed a fish meal-based diet or a zygomycete-based diet (Paper I). The findings on growth performance were consistent with previous report of a study in which a mycelium based biomass diet replaced a fish meal-based diet (Bankefors *et al.*, 2011). However further studies are required to investigate the growth performance of fish fed graded levels of the biomass based diet.

6.2. Zygomycete ingredients in the diets enhance chitinolytic activity and disturb nutrient uptake and intestinal integrity

Chitinases and their role have been observed in the GI-tract of dover sole, sablefish, shortspine thornyhead and rockfish (Gutowska *et al.*, 2004) and these enzymes are correlated to chitin content in the diet (Fange *et al.*, 1979). In the present study (Paper I), there was an indication of a correlation between diet and chitinase activity in the gut tissues of Arctic charr. The zygomycete-based diet (FZ) fed to the fish greatly affected exo-chitinase activity in the different regions of GI-tract. The fish meal-based diet (FM) showed greater endo-chitinase activity in the gut tissues of the fish. It is hypothesized that the differences in chitinolytic activity in the gut tissues of Arctic charr may reflect their capacity to digest and absorb chitin in the diet. It has also been claimed that feeding a chitin-rich diet to cod fish enhances the chitinase activity (Danulat, 1986). Moreover, chitinolytic activity is reported to remain unaffected in the GI-tract of rainbow trout fed 10% dietary chitin (Lindsay *et al.*, 1984). In juvenile shrimp, increasing dietary levels of chitin greatly reduce chitinase activity in the GI-tract (Fox, 1993). Therefore, further studies are needed to identify differences in chitinolytic activity and their impact on digestive physiology when feeding different sources of microbial biomass.

The chitin molecules produced as a result of chitinase activity show variation in the rate of absorption in dover sole, sablefish, shortspine thornyhead, rockfish, juvenile shrimp and rainbow trout (Gutowska *et al.*, 2004; Lindsay *et al.*, 1984; Peres, 1981). In general, nutrient absorption depends on the rate at which chitin molecule come into contact with the absorptive epithelium. In Paper I, the apparent differences observed in paracellular permeability (P_{app}) in regions of the GI tract suggested that the test diets produced different effects in the different regions. The higher P_{app} value in the distal intestine of Arctic charr fed the zygomycete diet could be due to disturbed intestinal integrity and leakage in the region, and a sign of impaired intestinal barrier function. The intestinal barrier function may be reduced by exposure of the intestinal mucosa to the zygomycete ingredient, which can act as an irritant (Mydland *et al.*, 2009).

It has been demonstrated that dietary chitosan is most likely responsible for interfering with nutrient absorption in the intestinal epithelium (Deuchi *et al.*, 1994). The higher lysine uptake in the distal intestine induced by the zygomycete-based diet and the lack of response in the proximal intestine suggest a negative effect on nutrient absorption of this diet. This indicates disturbed integrity in the distal intestine resulting in higher P_{app}, which may allow increased diffusional passage of nutrients across this intestinal region. Taken together, the results presented in this thesis indicated that the

zygomycete-based diet influenced chitinolytic activities differently in different regions of the GI tract and exerted negative effects on the paracellular permeability of nutrients in the intestinal tissues of Arctic charr.

6.3. Zygomycete-based and fish meal-based diets cause no difference in metabolic response in Arctic charr

In this thesis, we explored a zygomycete biomass based diet as an alternative feed ingredient using highly advanced ^{1}HNMR technology (Paper II). PCA is a unsupervised method that is commonly used to identify how one sample is different from another, which variable contributes to significant differences and whether those variables are correlated or independent from one another (Wishart, 2008). The PCA score scatter plot demonstrated no apparent separation or clear trend in the clusters of the diets (Paper II). OPLS-DA, which is a supervised method, is used to determine and increase the separation between groups of observations (Wishart, 2008). The OPLS-DA model revealed that it could discriminate among the FZ, FM and ST diets due to differences in the spectral data (Paper II). The findings demonstrated that the zygomycete-based and fish meal-based diets showed no difference in metabolic profile (Paper II) and these results were supported by the growth response of the fish (Paper I). Most of the metabolites obtained when feeding these two diets to Arctic charr were consistent with previously obtained metabolic profile fed fish meal and biomass based diets to Atlantic salmon (Bankefors *et al.*, 2011; Castejon *et al.*, 2010). It has been demonstrated using ^{1}HNMR spectroscopy that plant and bacterial protein meal can replace up to 25% of fish meal in the diet without affecting the growth rate in rainbow trout and Atlantic salmon (Storebakken *et al.*, 2004; Perera *et al.*, 1995).

6.4. Metabolic response in Arctic charr and tilapia fed different levels of wheat starch

The PCA scatter score plot indicated that feeding different levels of wheat starch to tilapia did not result in any tendency for clustering. In Arctic charr there was a clear tendency for clustering and clusters of the diets were distinct from each other (Paper III). These findings imply a significant effect on metabolite clusters related to starch level in the diet of Arctic charr. OPLS-DA revealed discrimination of components of pre-defined classes suggested diet effects on the metabolic profiles of Arctic charr and tilapia. In Arctic charr, discrimination of glucose and choline were prominent in the control diet and discrimination of asparagine, lactate and 3-aminobutyrate were prominent in

the diets containing 10% and 20% wheat starch. This suggests that in Arctic charr, there is a partial or negligible influence on the metabolism of dietary starch. These findings may in relation to carnivorous fish species have lower utilisation efficiency of carbohydrates (NRC, 2011; Arockiaraj *et al.*, 1999). In accordance with described, lower growth response and glucose metabolism blunt snout bream fed the highest carbohydrates/lipid ratio (Li *et al.*, 2013b). It is believed that if fish are not supplied with an appropriate amount of dietary carbohydrates, they metabolise other nutrients, *e.g*. proteins and lipids, for their energy needs (Wilson, 1994). It is reported that deamination of aspargine is result of gluconeogenesis in rainbow trout (French *et al.*, 1981). In tilapia, discrimination of the metabolites ADP, creatine, UDP-glucuronate and O-phosphocholine was prominent in 20% wheat starch samples, which indicates more efficient metabolism of dietary starch. It has been demonstrated that tilapia metabolise high dietary starch using hepatic enzymes involved in the glycolysis pathway (Azaza *et al.*, 2013). Carbohydrates are metabolised by glycolysis or the pentose phosphate pathway, leading to generation of energy transfer molecules in fish (Polakof *et al.*, 2012; Richard *et al.*, 2006). Also, dietary carbohydrates could depress the increase rate of amino acid metabolism and utilisation by gluconeogenic pathways in salmon fish (Sanchez-Muros *et al.*, 1996). Certain fish species such as tilapia, channel catfish and grass carp possess the ability to utilise up to 40% dietary starch (Lin, 1991; Luquet, 1991; Satoh, 1991). Overall, the different levels of wheat starch fed to Arctic charr and tilapia resulted in variation in metabolic profiles.

6.5. Apparent digestibility and amylase activity in relation to dietary starch levels

Digestibility and enzyme activity in response to dietary carbohydrates differ between fish species and usually depend on level of dietary intake, source and composition of diet (Enes *et al.*, 2011; Peres *et al.*, 1996). In this thesis, Arctic charr, Eurasian perch and tilapia fed diets containing different amounts and sources of carbohydrates responded differently (Papers I-IV). Apparently, each fish species possesses specific properties which affect its capacity to utilise and metabolise carbohydrates. Importantly, within fish species the enzymatic, digestive and metabolic characteristics in response to feeding starch were not influenced by inclusion level (Papers III and IV). However, the metabolic characteristics and amylase activities showed variations between fish species fed similar inclusion levels. The findings of low digestibility of starch in Arctic charr (Paper IV) support previous findings in other salmonid fish species (Krogdahl *et al.*, 2005; Hemre *et al.*, 1995). The lower digestibility of starch

may be due to carnivorous fish species possess lower ability to utilise dietary carbohydrates than omnivorous and herbivorous fish (Enes *et al.*, 2011; Enes *et al.*, 2006; Rust, 2002). In Eurasian perch, the higher digestibility of the 20% wheat starch diet than the other diets (Paper IV) indicates an impact of starch inclusion level on the efficiency of utilisation. The reason for this is unknown and merits further investigation. Moreover, further work is needed to establish the most appropriate amount of starch of varying origin, in native and processed form, in the diet of Arctic charr and Eurasian perch.

7. General conclusions

- Arctic charr showed significant chitinolytic activity in tissues of the gastrointestinal tract and feeding a zygomycete-based diet (rich in chitin) resulted in increased chitinolytic activity. However, inclusion of zygomycete biomass in the diet disturbed intestinal primary barrier functions and negatively influenced nutrient uptake and intestinal integrity in the fish.
- A zygomycete-based diet and a fish meal-based diet fed to Arctic charr did not exhibit differences in metabolic response analysed using ^{1}H NMR technique.
- ^{1}H NMR metabolomics approach revealed differences and apparent variations in metabolic profiles in liver tissues of tilapia and Arctic charr fed different starch level.
- Eurasian perch and Arctic charr possess starch digestive capacity, but with markedly higher starch digestibility in Eurasian perch. Differences in starch digestive ability were supported by α-amylase activity in the intestinal region.

8. Implications and future research

The data presented in this thesis on metabolism in Arctic charr, tilapia and Eurasian perch fed dietary zygomycete biomass and wheat starch in their diet can be of great value in practical diet formulation for these fish species. Moreover, the information on enzyme activity, digestibility and growth performance can be practically applied to improve utilisation of carbohydrates included in the diets of these fish species. The information generated will be useful in the search for replacements for fish meal using cheap, renewable alternative resources in the formulation of fish diets for sustainable aquaculture production.

Future studies are needed in order to:

- Evaluate the impact of different levels of dietary inclusion of microbial biomass on the digestive physiology and growth performance of Arctic charr, tilapia and Eurasian perch.

- Investigate the influence on growth characteristics and metabolic activities of inclusion of different dietary levels and sources of carbohydrates in aquafeeds for different fish species.

- Formulate fish diets from chitin rich aquatic organisms and to evaluate their impact on feed intake, adaptation, metabolism and growth performance in different fish species.

9. Smältbarhet och omsättning av kolhydrater i fisk

Akvatiska produkter utgör en väsentlig del av människors föda i stora delar av världen och de bidrar påtagligt till intaget av högvärdigt protein. Fisk och produkter från fisk kommer från viltfångad fisk och från odling av fisk i olika vatten. Under de senaste fyra decennierna har den globala odlingen av fisk och andra akvatiska organismer ökat med cirka 2,9 % per år.

Tillgång till foder och kostnader för foder utgör en av de största utmaningarna för en långsiktigt uthållig odling av fisk. Fiskindustrin söker därför aktivt efter fodermedel som kan användas för produktion av billigt fiskfoder. Under lång tid har fiskmjöl varit den dominerande proteinkällan i fiskfoder och fiskolja har ingått till betydande del för att höja fodrets energiinnehåll. Ökad efterfrågan och ökade kostnader för fiskmjöl och fiskolja utgör uppenbara hinder för en långsiktigt uthållig ökning av fiskodling, och det behövs alternativa fodermedel som kan användas som ersättning.

Möjliga alternativa fodermedel som kan användas som ersättning kan variera i ursprung, och kan komma från växter, djur eller mikroorganismer. Vanligtvis är kolhydrater billigare som energikälla än protein och fett. Emmelertid uppvisar fiskar en varierande förmåga att smälta och omsätta olika beståndsdelar i fodret, detta gäller särskilt kolhydraterna. Studier visar att smältbarhet och omsättning av olika fodermedel beror på fiskart, men också på ursprung, inblandningsnivå i fodret, och eventuell behandling av fodermedlet. Ingående kunskap om fiskars förmåga ett utnyttja kolhydrater i fodret är en viktig förutsättning för att framgångsrikt kunna utforma väl fungerande fiskfoder.

Fokus för denna doktorsavhandling har varit att öka kunskapen om fiskars förmåga att smälta och omsätta kolhydrater från cerealier, i form av stärkelse från vete, och från mikrosvampar, i form av kitin. I de foder som användes ingick båda stärkelse och kitin i obehandlad form, för att kunna

bedöma de studerade fiskarnas förmåga att utnyttja dessa kolhydrater i nativ form.

Det övergripande målet med denna avhandling var att studera digestion och omsättning av kolhydrater i röding (*Arctic charr*) och abborre (*Eurasian perch*). För att åstadkomma detta genomfördes försök *in vivo* med fiskar som utfodrades med olika foder, och genom insamling av vävnadsprover för analys av enzymaktivitet, tarmfunktion och koncentration av metaboliter.
De specifika målen var att:

- Undersöka förmågan hos röding att utnyttja foder med högt innehåll av kitin/kitosan genom att bestämma den kitinolytiska aktiviteten i tarmvävnad och absorptionen av aminosyror genom tarmvävnad hos fiskar som utfodrats med ett foder baserat på fiskmjöl, med eller utan inblandning av biomassa från zygomycet (*Rhizopus orycae*).

- Utvärdera inverkan av utfodring med biomassa från zygomycet på tarmens barriärfunktion hos röding.

- Jämföra metabolitprofilen i lever hos röding som utfodrats med ett foder baserat på fiskmjöl, ett foder baserat på zygomycet och ett kommersiellt foder.

- Utvärdera metabolitprofilen hos röding som utfodrats med nativ vetestärkelse, i jämförelse med metabolitprofilen hos tilapia (*Oreochromis mossambicus*).

- Undersöka inverkan av olika inblandningsnivåer av nativ vetestärkelse på smältbarhet och amylasaktivitet hos röding och abborre.

De genomförda studierna visar att tarmvävnad hos röding uppvisar betydande kitinasaktivitet, både endo-och exo-kitinasaktivitet. Studierna visar också att kitinasaktivitetens fördelning längs tarmen hos röding varierade mellan endo- och exo-kitinas. Endo-kitinasaktiviteten i magsäcksvävnad och i vävnad från den distala delen av tarmen var flera hundra gånger högre än exo-kitinasaktiviteten i magsäcksvävnad. Den högsta exo-kitinasaktiviteten uppmättes i vävnad från den distala tarmen. Utfodring av röding med det zygomycetbaserade fodret resulterade i högre kitinolytisk aktivitet i tarmvävnad än utfodring med det fiskmjölsbaserade fodret. Röding som utfodrats med det zygomycetbaserade fodret uppvisade störd tarmfunktion och ett ökat upptag av aminosyran lysin i den distala delen av tarmen, men inte i den proximala delen av tarmen.

Metabolomik, baserad på kärnmagnetisk resonans (^{1}H-NMR), kunde inte påvisa några skillnader I metabolitprofiler i levervävnad från röding som utfodrats med ett zygomycetbaserat foder respektive ett fiskmjölsbaserat foder.

Vetestärkelse påverkade inte aktiviteten hos α-amylas i tarmvävnad från röding och abborre. Generellt, var aktiviteten för α-amylas korrelerad till de skillnader som uppmättes för stärkelsens smältbarhet. Den skenbara fekala smältbarheten för råprotein, stärkelse, råfett och energi skiljde sig mellan fiskarter med i genomsnitt högre värden för samtliga variabler hos abborre jämfört med röding. Inom fiskart hade fodrets innehåll av vetestärkelse ingen påverkan på den skenbara fekala smältbarheten för torrsubstans, råprotein, råfett och energi.

Det metabola svaret på utfodring med vetestärkelse hos röding och tilapia studerades med metabolomik baserad på ^{1}H-NMR. Resultaten visar att metabolismen hos tilapia påverkades av utfodring med vetestärkelse, medan metabolismen hos röding påverkades partiellt eller försumbart. Resultaten tyder på artspecifika skillnader i det metabola svaret på utfodring med vetestärkelse.

Ytterligare studier behöver genomföras för att:

- Utvärdera inverkan på tarmfysiologi och tillväxt hos röding, abborre och tilapia av gradvis ökande inblandning av mikrobiell biomassa i fodret.

- Undersöka inverkan på tillväxtmönster och metabol aktivitet hos olika fiskarter vid utfodring med olika kolhydratkällor och med gradvis ökande inblandningsnivå.

- Utforma fiskfoder baserat på kitinrikt avfall från vattenbruk och utvärdera deras påverkan på foderintag, tillvänjning, omsättning och tillväxt hos olika fiskarter.

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Acknowledgements

In the name of Allah Almighty, the most Gracious, the most Beneficent and the most Compassionate. The One God, who blessed and loadeth me with countless benefits and made me capable to achieve my goals.

The financial support of Islamic Development Bank, Jeddah, Kingdom of Saudi Arabia, under the PhD Merit Scholarship Programme is greatly acknowledged and appreciated. Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala, Sweden is highly acknowledged for providing platform and necessary facilities for academic and research.

Professor **Jan Erik Lindberg**, main supervisor, I would like to express the deepest appreciation and thanks for his great interest and valuable comments in the papers and in this thesis.

Torbjörn Lundh and **Ali Ata Moazzami,** co-supervisors, I am grateful for their help in academic and research.

Margareta Emanuelson, Head of the Department of Animal Nutrition and Management, gratitude for her nice and kind response during discussion.

Anna-Greta Haglund and **Margareta Norinder**, sincere thanks for their help and immediate response, whenever I needed!

Markus Langeland and Aleksandar Vidakovic, thanks for their help in experiments and friendly discussion. Also thankful to Alexander for providing nice pictures of fish species in this thesis.

Kristina Andersson, thanks and grateful for help in statistics and discussions.

Mary McAfee, thanks and grateful for help in English correction in the papers immediately especially for thesis in limited time.

I take to this opportunity to thank all my co-authors of the papers, Professor Kristina Sundell, Erik Sandblom, Henrik Sundh, Eva Brännäs, and Anders Kiessling, for their contribution and valuable input.

David Morrison, Department of Biomedical Sciences and Veterinary Public Health, I am extremely thankful for his continuous support and enthusiastic help in statistics and English correction in the papers in this thesis. He is kind hearted, great and always ready to help me immediately, whenever I needed!

It is great pleasure to me work and study in such a great atmosphere provided help, support by present and former colleagues/friends; I would like to express my sincere thanks to: Malavanh, Daovy Kongmanila, Sath, Hao-Yu Liu, Sara Ringmark, Haue, Ngoc, Thi Da, Jenny, Cecilia Kronqvist, Tham, Maria Neil, Hans Pettersson, Anna Johansson, Mazyar Arash Nia, Rouzbeh Kiyani, Emma Ivarsson and Aahana Shrestha.

I would like to express the deepest appreciation for Pakistani family friends here in Uppsala. I am grateful to my family friends Sussane, Katarina Olympian, Kerstin de Verdier and Shakoufeh Karimi for their friendly discussions and kindness shown to my family.

I express the deepest love for my father, mother, brothers and sisters whose prayers, love and care made me to achieve this position. I never forget my sister **Hakimzadi Wagan** for her love, care and encouragement since childhood.

My Little world; I have no words to express feelings of love for my daughter Little princess **Samreen**, son prince **Ayan** and loving and caring husband **Shahid Hussain Abro** who brought countless happiness in my life. Samreen's sweet chatting and Ayan's smile make me extremely comfortable and feel in heaven!